IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	J. Nelson	Group Art Unit:	1637
Serial Number:	10/770,657	Examiner:	K. R. Horlick
Filing Date:	February 3, 2004	Docket No.:	PB0308
Title:	cDNA Amplification for Expression Profiling		

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Declaration of Prior Invention in the United States or in a NAFTA or WTO Country to Overcome Cited Patent or Publication (37 C.F.R. § 131)

1. This declaration is to establish completion of the invention in this application in the United States, as a date prior to December 31, 2002, that is the effective date of the United States Patent No. 6,977,153 by Kumar, et al. that was cited by the Examiner.

2. The person making this declaration is the inventor.

3. To establish the date of completion of the invention of this application, a copy of the initial invention disclosure by the inventor to the employer is submitted as evidence; the date has been obliterated. This document (Internal Technology Record, or ITR, entitled cDNA Amplification for Expression Profiling), has a date prior to December

31, 2002, thus, invention claimed in this application was made prior to December 31, 2002, which is a date earlier than the effective date of the reference.

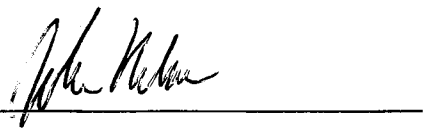
4. From the accompanying document, it is clear that the inventor had constructive reduction to practice of the invention in this application before December 31, 2002.

5. This declaration is submitted prior to final rejection.

As a person signing below:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole inventor: **John R. Nelson**

Inventor's signature: 

Date: Nov 7, 2006

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STRICTLY CONFIDENTIAL

Internal Technology Record

Amersham Biosciences

document no.
ITR

To submit: Obtain the required signatures below including that of your local Science Director who will send the signed form to the Ideas Screening Manager for logging into the system.

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Archive: By Ideas screening administration. Original to be filed for 10 years in the Ideas database.

title of idea/invention		
cDNA amplification for expression profiling		
author(s) 1 John Nelson	signature	date (ccyy-mmm-dd)
2		
tel no		
1 732-980-2850	2	
R&D site Piscataway		
read and witnessed TANY MAMONE	signature	date (ccyy-mmm-dd)
approval for registration TONY MURRAY	signature (Staff/Senior Scientist)	date (ccyy-mmm-dd)
final approval	signature (Science Director)	date (ccyy-mmm-dd)

Help texts
Help texts that are built in to this form are written with *hidden text* in blue on yellow background.
To view the help texts, go to Tools/Options/View and tick 'Hidden text'.

1. Key words associated with this idea/invention

Amplification, expression profiling

2. Specify any features of this idea/invention that offer improvements or advantages over existing techniques/applications/products

There are a number of patents in this field, but none of them encompass this idea.

3. Commercial value/strategic fit of idea to the company (if known)

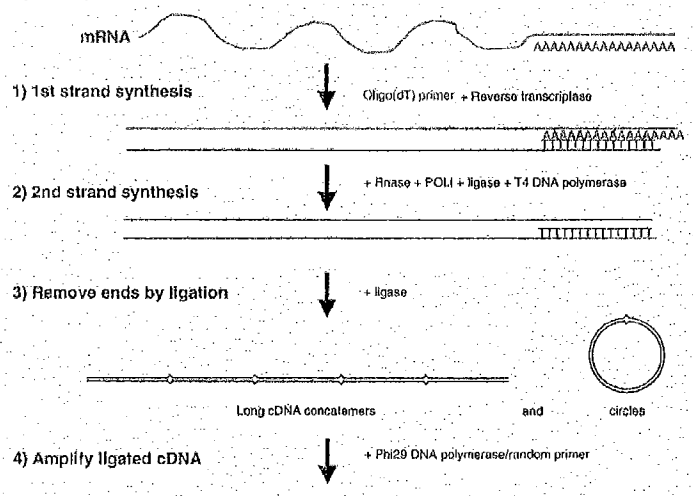
4. Laboratory notebook/background references

5. Attachments

6. Full description of idea/invention and proposed method for putting it into practice.

1) We have developed a new cDNA amplification method. The mRNA is first converted to double stranded cDNA, and then this cDNA is blunt-end ligated to form circles (or maybe, but unlikely,concatemers). This is then amplified using a Phi29 DNA polymerase based random primed strand displacement amplification method (see figure 1). The resulting product can then be used to make probe for gene expression studies.

Figure 1)



I believe we should patent this workflow.

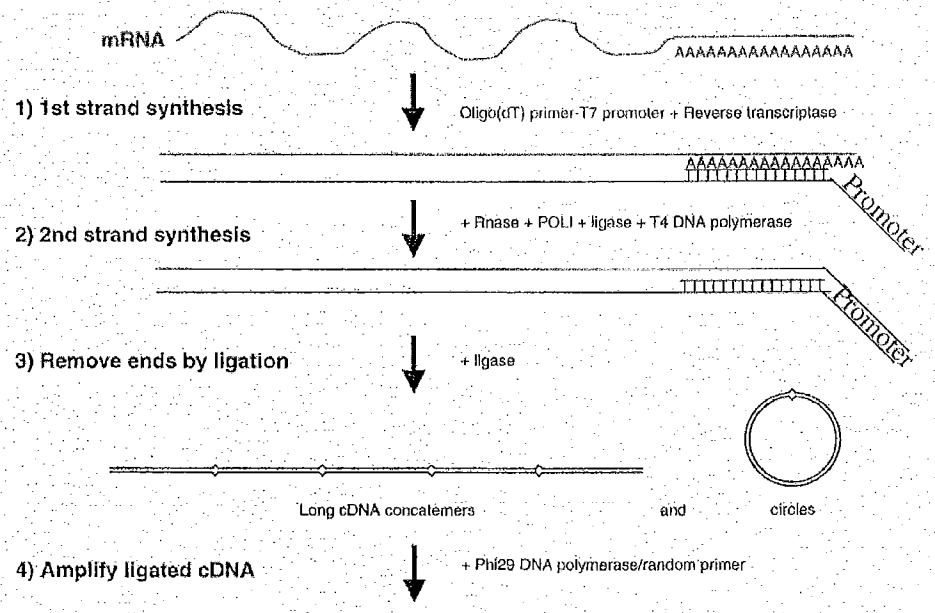
The Lizardi patent has claims specific for using concatenated cDNA (formed by ligation of cDNA) as template for strand displacement amplification, but the claims make no mention of using circularized cDNA. The method being developed here is at least partially designed to allow for gene expression analysis from small numbers of cells. The cDNA made from this type of sample will have a very low concentration, which will prevent concatenation. Instead the cDNA will circularize. This idea uses circularized cDNA as the input template for strand displacement amplification.

I recommend submitting an Amersham Biosciences patent with claims specific for strand displacement amplification of cDNA that has been ligated to form circles (monomer size) either with or without concatemers. This should cover both random primed and specific primed amplification methods. Also, it should included the following ideas.

2) This idea has another aspect involving the particulars of the workflow and use of the product. A number of patents in the field of gene expression study use an RNA polymerase promoter which is attached to the 5' end of the primer used to generate the first strand during cDNA synthesis. After second strand synthesis the now

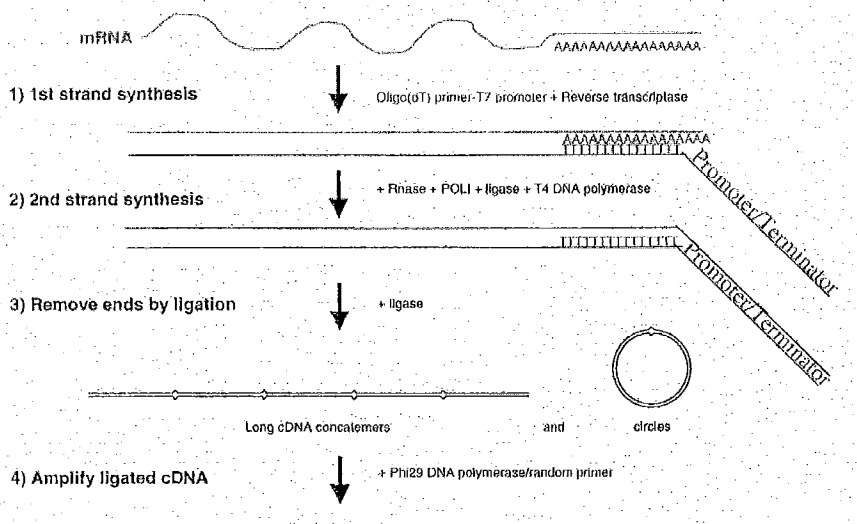
double stranded cDNA is transcribed to make an antisense RNA transcript which can be used for expression studies (RNA quantification). One drawback to this workflow is that the transcription reaction can provide only limited amplification. I propose using the promoter-tagged cDNA as a template for cDNA ligation, ligating to form either concatemers or circles or a mixture of both. This material could then be amplified by strand displacement amplification (see figure 2). The amplified material would then be transcribed for use in quantification studies. This method could provide for a considerable amplification, greater than what is currently available. Additionally, the resulting RNA would be antisense, which could be advantageous.

Figure 2)



3) This idea has one additional aspect. The strand displacement amplification of circularized or concatenated cDNA will generate end-to-end concatemers of the input template cDNA units. These units will each have a separate RNA polymerase promoter. In order to prevent overlapping transcription from these concatenated units, an RNA polymerase terminator sequence can be included upstream of the promoter sequence used in the primer for first strand cDNA synthesis (see figure 3). This terminator will prevent over-amplification of cDNA's resulting from repeated transcription by read-through transcription from upstream promoters.

Figure 3)


mRNA
AAAAAAAAAAAAAAAA
1) 1st strand synthesis
Oligo(dT) primer-T7 promoter + Reverse transcriptase
AAAAAAAAAAAAAAAA
2) 2nd strand synthesis
+ Rnase + POLI + ligase + T4 DNA polymerase
Promoter/Terminator
Promoter/Terminator
3) Remove ends by ligation
+ ligase
Long cDNA concatemers
and
circles
4) Amplify ligated cDNA
+ Phi29 DNA polymerase/random primer